Neuroscience Letters 450 (2009) 37–39

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Altered neuronal densities in sexually dimorphic structures: Comparable effects from perinatal magnetic fields with nitric oxide synthase inhibitors and postnatal hypoxia

Karli K. McDonald [1], Michael A. Persinger *

Behavioural Neuroscience Program, Laurentian University, Ramsey Lake Road, Sudbury, Ont., Canada P3E 2C6

ARTICLE INFO

Article history:
Received 29 August 2008
Received in revised form 5 November 2008
Accepted 12 November 2008

Keywords:
Sexual dimorphism
Hypoxia
Magnetic fields
Nitric oxide
L-NAME
Ventromedial hypothalamus
Bed nucleus of stria terminalis
Perinatal exposure
Rat

ABSTRACT

Cytometry of the neuronal density within four sexually dimorphic nuclei was completed for adult rats that had been perinatally exposed to 0.5 Hz, 5–10 nT magnetic fields or sham conditions while their mothers drank tap water containing the nitric oxide synthase (NOS) inhibitor L-NAME or only tap water. One week after birth the rats were rendered hypoxic for 1 min or served as controls. Exposures to either the magnetic field or the NOS inhibitor reduced the numbers of neurons within the bed nucleus of the stria terminalis by about 25%, whereas exposure to either the hypoxia or magnetic fields resulted in comparable decreases in cell numbers within the ventromedial nucleus (dorsomedial part). For comparison males had 15% fewer neurons in these nuclei compared to females. The effect sizes for the interactions involving the perinatal exposure for 8 days to the magnetic fields were comparable to the magnitudes of those associated with 1 min of hypoxia 1 week postnatally. These results show the sensitivity of specific structures of the developing brain to interactions between subtle environmental variables.

© 2008 Elsevier Ireland Ltd. All rights reserved.

The developing brain is extremely sensitive to subtle stimuli to which the adult organism is oblivious. Prenatal exposure for 48 h to a 5–10 nT 0.5 Hz magnetic field designed to simulate natural geomagnetic pulsations significantly reduced litter sizes [7]. St-Pierre et al. [24] found that rats exposed perinatally from −2 to +5 days to a 7-Hz, 5–10 nT square-wave magnetic field showed twice the mortality at weaning to chemically induced seizures compared to rats that had been exposed to weaker or stronger fields. In other experiments [26] the behaviours of rats exposed to this frequency with intensities around 50 nT and the nitric oxide (NO) precursor L-arginine during the same perinatal period suggested the magnetic fields interacted with the effects of NO. Increased testicular and brain weights were noted in these animals even as older adults [28].

Although prenatal or perinatal exposures to more intense extremely low frequency (ELF) magnetic fields have been shown previously to affect the cell density of the sexually dimorphic medial preoptic nucleus [20] and levels of NO [11,29,1], there have been no quantitative assessments of the weaker (5–10 nT) fields. In the present experiments we selected four sexually dimorphic [15] structures: the bed nucleus of the stria terminalis (BNST), the medial amygdaloid nucleus (MeA), the medial preoptic nucleus (MPO), and the ventromedial nucleus (VMN) of the hypothalamus for examination. These structures exert powerful effects upon reproduction, food consumption, and gender-specific behaviours [15].

Because of the importance of NO pathways in oxidative stress such as hypoxia, we also discerned the effects of 1 min of exposure to hypoxia on postnatal day 7, a procedure that produces changes in adult learning [8,4]. We also wanted to directly compare the effects of a subtle stimulus, prolonged environmentally comparable magnetic field exposures, to a single, brief and physiologically extreme event, such as 1 min of hypoxia. This would allow quantitative comparisons of the two conditions as well as potential interactions within the same study.

Brains from a total of 54 (37 male, 17 female) Wistar albino rats that been collected by the second author were selected by the first author from a much larger sample for examination. The brains had been removed within 5 min after decapitation when the rats were about 120 days of age. Approximately equal numbers of rats had been exposed to either a 5–10 nT, 0.5 Hz magnetic field or to a sham

* Corresponding author. Fax: +1 705 675 4824.
E-mail address: mpersinger@laurentian.ca (M.A. Persinger).
[1] Present address: Department of Medicine, Centre Hospitalier de l'Universite de Montreal, 1560 Sherbrooke Est., Pavillion Mailloux M-5237, Montreal, Que., Canada H2L 4M1.

0304-3940/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2008.11.029

field (<1 nT) between −2 and +5 days of birth [7] while their mothers drank either tap water or water containing 1 g/L of the NOS inhibitor L-NAME [16].

The mothers and pups had been housed in standard plastic cages [7] within one of two aluminium racks each of which were 112 cm × 125 cm and wrapped with 72 turns of 30 gauge wire (width = 13 cm). One coil received input from a Heath Schlumberger function generator while the other served as the sham area. In two of the four experimental blocks the previously activated coil served as the sham area. The 5 nT (central to the coil) to 10 nT (closer to the coil) intensity of the generated 0.5 Hz magnetic fields, was verified continuously by the sensor of a MEDA FM 300 Vector magnetometer positioned in its own identical plastic cage. The static geomagnetic field intensities within the *X* (direction of applied field), *Y*, and *Z* (vertical) coordinates were 13,800–14,200 nT, 18,850–21,000 nT, and 39,200–41,800 nT, respectively.

At 7 days of age the rats served as controls or were rendered hypoxic for 1 min with either CO_2 or N_2. In all cases the rats' reflexes returned to normal within 4 min. Because in unpublished analyses we found no difference in the effects of the two gases and to maximize the sample size, rats were classified as normal or hypoxic.

Every 20th section of the 10 μm paraffin-embedded sections of the brains was stained with toluidine blue 0. Under 400× the numbers of neurons (defined by a least a portion of the nucleus present) were counted within a 6 × 6 grid within several fields separately in the left and right sides of the BNST (lateral, medial anterior, and ventral subdivisions), MeA nucleus (dorsomedial, central, and ventral lateral parts), MPO nucleus, and VMN (dorsomedial, central, and ventrolateral divisions). The positions and structures were verified by Paxinos and Watson [22]. The average of the two non-adjacent sections per subdivision was obtained. Inter-rater reliability based upon 15 randomly selected sections was >0.90.

The primary statistical design was a three-way analysis of variance as a function of treatments for magnetic fields (sham vs. 0.5 Hz, 5–10 nT), NO-inhibition (water vs. L-NAME), and hypoxia (no vs. yes). Sex was employed as a covariate rather than a main effect in order to increase the sample size (n = 6–7/group) for the eight groups. The numbers of neurons per unit area were analyzed for the left and right of each of the four structures separately. *Post hoc* analyses involved Tukey set at $p < .05$. η^2, the amount of variance in neuronal numbers explained by the treatments, was calculated for each of the most significant effects in order to compare effect sizes. All analyses involved SPSS software on a VAX 4000 computer.

The strongest and most statistically significant effects were primarily *two-way interactions* (all d.f.s = 1, 45) between NOS-inhibiting, hypoxia, and/or magnetic field exposures. This means there were 12–14 rats for each of the four groups. The statistically significant interaction between the NOS-inhibiting treatment and hypoxia found for the right ($F = 5.25$, $p < .05$; $\eta^2 = 6\%$ variance explained) and left ($F = 5.54$, $p < .05$; $\eta^2 = 6\%$ variance explained) dorsomedial division of the VMN was shown by *post hoc* analysis to be due to the ameliorating effects of hypoxia plus NOS inhibition.

There was also a significant interaction ($F = 4.47$, $p < .05$; $\eta^2 = 9\%$) between magnetic field exposure and NOS-suppression for numbers of neurons within the lateral division of the right BNST. According to *post hoc* analysis the interaction was due to the fewer numbers of neurons subsequent to the magnetic field exposure compared to sham field-exposed rats. This decrement was not evident in the rats that had received the NOS inhibitor while being exposed to the field.

The third statistically significant interaction ($F = 6.10$, $p = .01$; $\eta^2 = 9\%$) was between the hypoxia and magnetic field conditions for the right dorsal medial division of the VMN only. *post hoc* analysis indicated this interaction was due to the diminished numbers of neurons in the dorsomedial division of the right VMN which was attenuated if the rats had been exposed previously to the magnetic field. All main effects were affected by these interactions and all other interactions were not statistically significant.

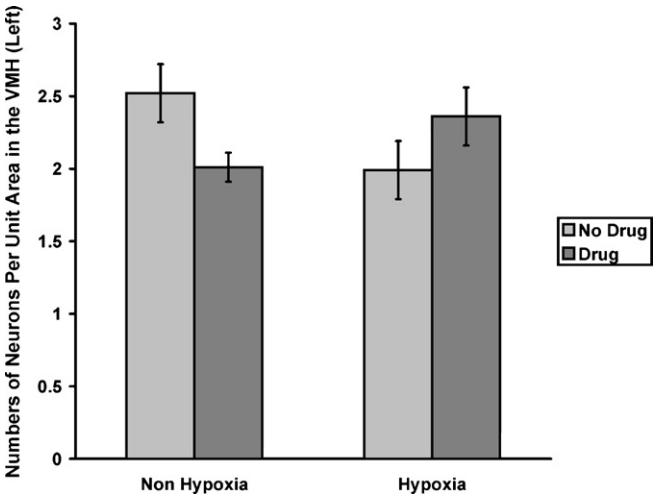

Fig. 1. Numbers of neurons per unit area within the ventromedial nucleus (dorsomedial part) of the hypothalamus of adult rats after perinatal exposure to the effects of L-NAME (drug) through their mothers' water supply or to tap water (no drug) and 1 min of hypoxia at 7 days after birth. Vertical bars indicate standard errors of the mean (S.E.M.s).

Perinatal exposures for 8 days (−2 to +5 days) to the weak magnetic field or to 1 min of hypoxia 1 week after birth both reduced numbers of neurons within the affected structures. However, these effects in large part were reversed to control levels or attenuated if the mothers had received the NOS inhibitor L-NAME. Portions of the VMN and the BNST were most affected by the interactions between perinatal NOS-suppression and postnatal hypoxia or the weak magnetic fields; the latter has been shown to enhance postnatal mortality to epileptic seizures [24]. The BNST and VMN strongly affect food intake and body weights in rats [23]. The BNST is a major source of vasopressin innervations of the lateral septum [6]. Subtle differences in either structure can change sexuality and irritability [14,30].

Although the mechanisms involving NO for the effects of both the hypoxia and magnetic fields employed in this study are not clear, direct effects on radicals and gaseous transmitters have been suggested for stronger (mT) magnetic fields [3]. ELF magnetic field-induced increases in NO can be diminished by NOS inhibitors as well as calcium channel blockers [12]. NO's relation to apoptosis is more clear [17] as is the role of apoptosis in sexual dimorphism in the brain [19]. On the basis of the changing asymmetry of measurements of astroglial nuclei within the left and right parasolitary nucleus on postnatal day 5 and day 15 Hu et al. [10] proposed that exposure to similar 0.5 Hz, 5–10 nT fields for −2 to +7 days around birth delayed the normal progression from asymmetry to symmetry during ontogenesis. The type of asymmetry can significantly affect an animal's strategies for foraging [13].

The results of this study demonstrated that "subtle" environmental stimuli affect the neuronal density of subsections of structures that exhibit differential expression of cell numbers within the sexes. One-minute of hypoxia at postnatal day 7 produces significant long-term changes in conditioning ability [8,4]. If the effects of the perinatal magnetic fields are similar in magnitude, then exposures to comparable frequencies and intensities within the man-made [27] or natural environment [5] may produce similar behavioural effects that may be significant for large populations (Figs. 1–3).

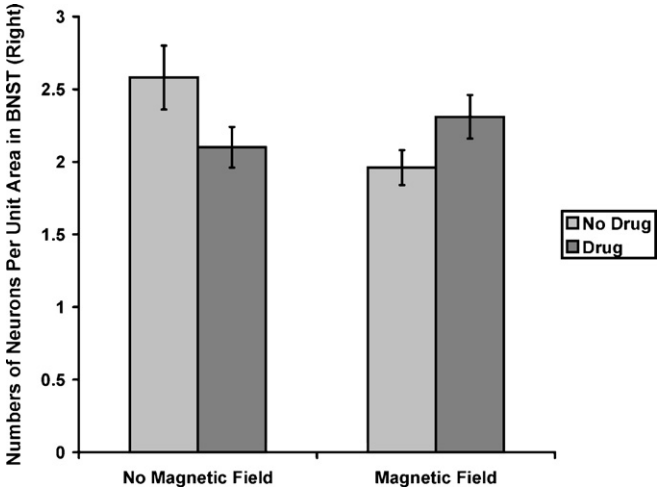

Fig. 2. Numbers of neurons within the bed nucleus (lateral division) of the stria terminalis for adult rats that had been exposed perinatally to either a sham field (no field) or 0.5 Hz, 5–10 nT magnetic field and to either tap water or drug (NO inhibition) in their mothers' water supply. Vertical bars indicate S.E.M.s.

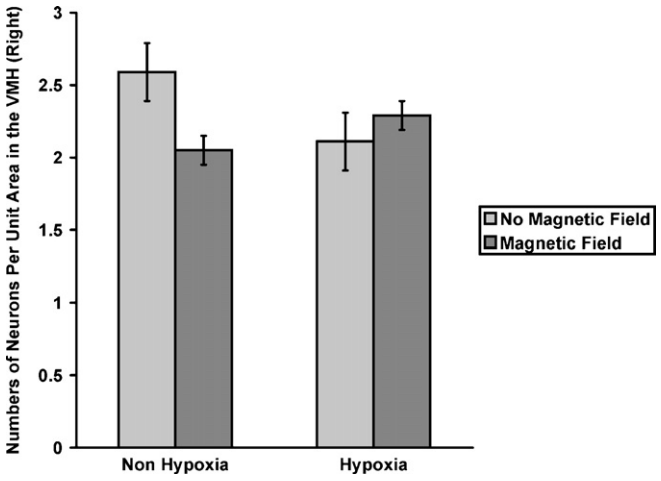

Fig. 3. Numbers of neurons within the ventromedial nucleus (dorsomedial part) of the hypothalamus of adult rats after perinatal exposure to a sham field or 0.5 Hz, 5–10 nT magnetic field and subsequent exposure at 7 days of age to either control (no hypoxia) or 1 min of hypoxia. Vertical bars indicate S.E.M.s.

There may be application to human behaviour. Weak but reliable correlations have been reported between geomagnetic activity (global averages between 1 and 50 nT) around birth and increased scores on psychometric measures of anxiety and temporal lobe lability [9,21]. In light of the accumulating literature concerning the global effects of geomagnetic activity on biological activity [2] and the presumed generation-to-generation effects upon social behaviour [25], subtle perinatal interactions may be considered relevant. However, the most powerful tool to examine these population-significant but correlation-weak effects is the experiment [18].

References

[1] M.Z. Akdag, M.H. Bilgin, S. Dasdag, C. Tumer, Alteration of nitric oxide production in rats exposed to a prolonged extremely low frequency magnetic field, Electromagn. Biol. Med. 26 (2007) 99–106.

[2] T.K. Breus, K.Y. Pimenov, G. Cornelissen, F. Halberg, E.V. Syutkina, R.M. Baevsky, V.M. Petrov, K. Orth-Gomer, T. Akerstedt, K. Otsuda, Y. Watanabe, S.M. Chibisov, The biological effects of solar activity, Biomed. Pharmacother. 56 (2002) 273s–283s.

[3] B. Brocklehurst, K.A. McLauchlan, Free radical mechanism for the effects of environmental electromagnetic fields on biological systems, Int. J. Radiat. Biol. 69 (1996) 3–24.

[4] J.C. Charette, L.S. St-Pierre, M.A. Persinger, Only 1 min of hypoxia from carbon dioxide during infancy in Rattus norvegicus scatters neurons in CA1 hippocampus and diminishes adult inhibitory responses to aversive stimuli (submitted for publication).

[5] N. Cherry, Schumann resonances: a plausible biophysical mechanism for human health effects of solar/geomagnetic activity, Nat. Hazards 26 (2002) 279–331.

[6] G.J. De Vries, R.M. Buijs, D.F. Swaab, Ontogeny of the vasopressingeric neurons of the suprachiasmatic nucleus and extrahypothalamic projections in the rat brain—presence of a sex difference in the septum, Brain Res. 281 (1981) 67–78.

[7] M.J. Dupont, G. Parker, M.A. Persinger, Reduced litter sizes following 48-h of prenatal exposure to 5–10 nT, 0.5 Hz magnetic fields: implications for sudden infant deaths, Int. J. Neurosci. 115 (2005) 713–715.

[8] A.J. Golanski, B.E. McKay, M.A. Persinger, Postnatal anxoia from CO_2 for 1 min produces sex-specific changes in contextual fear conditioning in rats, Percep. Motor Skills 93 (2001) 677–678.

[9] K.A. Hodge, M.A. Persinger, Quantitative increases in temporal lobe symptoms in human males are proportional to postnatal geomagnetic activity: verification by canonical correlation, Neurosci. Lett. 25 (1991) 205–208.

[10] J.H. Hu, G.H. Parker, M.A. Persinger, Perinatal exposure to 0.5 Hz, 5–10 nT magnetic fields delays the ontogeny of right–left asymmetry of astroglia numbers in the parasolitary nucleus: implications for sudden infant deaths (submitted for publication).

[11] A. Jelenkovic, B. Banac, V. Pesic, D.M. Jovanovic, I. Vasiljevic, Z. Prolic, Effects of extremely low-frequency magnetic fields in the brains of rats, Brain Res. Bull. 68 (2006) 355–360.

[12] J.H. Jeong, C. Kum, H.J. Choi, E.S. Park, U.D. Sohn, Extremely low frequency magnetic field induces hyperalgesia in mice modulated by nitric oxide synthesis, Life Sci. 78 (2006) 1407–1412.

[13] N.P. LaMendola, T.G. Bever, Peripheral cerebral asymmetries in the rat, Science 278 (1997) 483–486.

[14] P.D. MacLean, The limbic brain in relation to psychosis, in: P. Black (Ed.), Physiological Correlates of Emotion, Academic Press, NY, 1970, pp. 129–146.

[15] M.D. Madeira, A.R. Lieberman, Sexual dimorphism in the mammalian limbic system, Prog. Neurobiol. 45 (1995) 275–333.

[16] B.E. McKay, S.A. Koren, M.A. Persinger, Behavioral effects of combined perinatal L-Name and 0.5 Hz magnetic field treatments, Int. J. Neurosci. 113 (2003) 113–139.

[17] G. Melino, F. Bernassola, *S*-nitrosylation regulates apoptosis, Nature 388 (1997) 432–433.

[18] A.L. Michon, M.A. Persinger, Experimental simulation of the effects of increased geomagnetic activity upon nocturnal seizures in epileptic rats, Neurosci. Lett. 224 (1997) 53–56.

[19] J.A. Morris, C.L. Jordon, S.M. Breedlove, Sexual differentiation of the vertebrate nervous system, Nat. Neurosci. 7 (2004) 1034–1039.

[20] S. Mulligan, M.A. Persinger, Perinatal exposures to rotating magnetic fields "demasculinize" neuronal density in the medial preoptic nucleus of male rats, Neurosci. Lett. 253 (1998) 29–32.

[21] K.P. Ossenkopp, J.N. Nobrega, Significant relationship between perinatal geomagnetic activity and anxiety levels in females, Arch. Meteor. Geophys. Bioklim. 27 (1979) 75–90.

[22] G. Paxinos, C. Watson, The Rat in Stereotaxic Coordinates, 2nd ed, Academic Press, NY, 1986.

[23] B.L. Rollins, S.G. Stines, B.M. King, Role of the stria terminalis in food intake and body weights in rats, Physiol. Behav. 89 (2006) 139–145.

[24] L.S. St-Pierre, G.H. Parker, G.A. Bubenik, M.A. Persinger, Enhanced mortality of rat pups following inductions of epileptic seizures after perinatal exposures to 5 nT, 7 Hz magnetic fields, Life Sci. 81 (2007) 1496–1500.

[25] B.M. Vladimirsky, L.D. Kislovsky, Biophysics and history, Biophysics 43 (1998) 715–718.

[26] P.D. Whissell, M.A. Persinger, Development effects of perinatal exposure to extremely weak 7 Hz magnetic fields and nitric oxide modulation in the Wistar albino rat, Int. J. Dev. Neurosci. 25 (2007) 433–439.

[27] P.D. Whissell, M.A. Persinger, Emerging synergisms between drugs and physiologically patterned weak magnetic fields: implications for neuropharmacology and the human population in the 21st century, Curr. Neuropharmacol. 5 (2007) 278–288.

[28] P.D. Whissell, B.P. Mulligan, M.D. Hunter, H.P. Wu, G.H. Parker, M.A. Persinger, Developmental effects of 7 Hz, square wave magnetic fields and nitric oxide modulation of organ systems, Open Tox. J. 2 (2008) 7–12.

[29] N. Yasuko, A. Mori, R.P. Liburdy, L. Packer, Pulsed magnetic fields enhance nitric acid oxide synthase activity in the rat cerebellum, Pathophysiology 7 (2000) 127–130.

[30] J.-N. Zhou, M.A. Hofman, L.J. Gooren, D.F. Swaab, A sex difference in the human brain and its relation to transexuality, Nature 378 (1995) 68–70.